

Review

Isoflurane Preconditioning and Postconditioning in Multiple Organs Protection

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Abstract: As one of the most widely used volatile anesthetics, isoflurane preconditioning and postconditioning have been demonstrated to protect various organs, including heart, brain, spinal cord, lung, kidney, liver and intestine. However, the mechanisms of multiple organs protection by isoflurane have not been precisely clarified. Recent evidence from both *in vitro* and *in vivo* studies suggested that the proposed mechanisms related to the multiorgan protection by isoflurane include opening of adenosine triphosphate-regulated potassium channels, inhibition of mitochondrial permeability transition pore opening, activation of a diverse array of survival protein kinases, activation of endothelial and inducible nitric oxide synthase, release of reactive oxygen species, activation of anti-inflammatory response, up-regulation of anti-apoptotic factors, activation of transcription factors such as the hypoxia-inducible factor-1 and other pathways, which are reviewed in this article.

Keywords: anaesthetics, isoflurane, multiorgan protection, preconditioning, postconditioning

1. Introduction

Volatile anesthetic isoflurane has preconditioning and postconditioning effects in multiple organs. However, the involved underlying cellular and molecular mechanisms are still to be thoroughly investigated. The recent research progress in multiorgan protection by isoflurane and related mechanisms will be reviewed in this article.

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(Received January 10, 2013; Revised January 14, 2013; Accepted January 15, 2013; Published online January 28, 2013)

2. Effects of isoflurane on the protection in multiple organs

2.1. Isoflurane preconditioning

2.1.1. Myocardium

Isoflurane has direct protective properties against ischemic myocardial damage. In 1997, Cason *et al.* treated mice with precondition (15 min ischemia/30 min reperfusion) or isoflurane (15 min inhalation/15 min washout) before 30-min coronary occlusion [1]. They demonstrated that isoflurane had an ischemic preconditioning (IPC)-like effect, thus introducing the concept of anesthetic preconditioning (APC). Since then many well-designed animal studies have demonstrated that exposure to a volatile anesthetic significantly protects myocardium against subsequent ischemia/reperfusion (I/R) injury with better recovery of contractile function after ischemia and reduced infarct size [2-3]. Furthermore, the latest research showed that isoflurane late preconditioning was even extended to myocardial stunning, a sublethal myocardial injury [4-5].

The first clinical study using APC was published in 1999 [6], which demonstrated that administration of isoflurane prior to aortic cross clamping resulted in smaller postoperative release of creatine kinase MB and cardiac troponin I (cTnI). Subsequent clinical studies have been conducted in patients undergoing coronary artery bypass grafting (CABG) surgery. Tomai *et al.* [7] also reported that isoflurane suppressed the release of this enzyme in patients with compromised left ventricular function. In 2001, Haroun-Bizri *et al.* [8] showed that administration of isoflurane before cardiopulmonary bypass (CPB) improved postoperative cardiac output and decreased the degree of ST changes. Bignami *et al.* [9] performed a longitudinal study of 34,310 CABG interventions in 64 Italian cardiac surgical centers between 2002 and 2004. They estimated a risk-adjusted mortality ratio for each centre. The results showed that 30 days risk adjusted mortality was significantly reduced when volatile agents were used during cardiac surgery [9].

2.1.2. Brain and spinal cord

Numerous studies have confirmed that neurons preconditioned with isoflurane are more tolerant to ischemic injury [10-12]. For instance, Kapinya *et al.* demonstrated that preconditioning with 1.4% isoflurane for 3 hours at 0, 12, and 24 hours before middle cerebral artery occlusion (MCAO) reduced infarct volume in a rat focal cerebral ischemia. Blanck *et al.* also reported that pretreatment with 1.5% isoflurane for 30 min before 8 min of global ischemia could induce acute tolerance in a canine model of cardiac arrest. Exposure of primary cortical neuronal cultures to isoflurane before and with continued exposure during oxygen and glucose deprivation (OGD) also resulted in concentration-dependent attenuation of OGD-induced neuronal apoptosis. Isoflurane preconditioning can improve the long-term neurological outcome in neonatal and adult rats suffering from brain ischemia or hypoxia [13-15]. More interestingly, animal studies indicated that isoflurane preconditioning response can be attenuated by estradiol [16] and the beneficial effects of isoflurane preconditioning was only observed in ischemic male brain [17-18]. In addition, isoflurane was also found to have ischemic preconditioning effects on the spinal cord. Park *et al.* [19] first reported that isoflurane early preconditioning protected the spinal cord against neuronal damage in a dose-response manner in a rabbit model of transient spinal cord ischemia. Subsequently, Sang *et al.* [20] further demonstrated that repeated exposure to isoflurane induced delayed preconditioning against spinal cord ischemic injury in rabbits after 24 and 48 h but not 72 h. Moreover, other studies also confirmed that isoflurane-induced delayed preconditioning after spinal cord ischemia improved histopathological outcomes [21-22].

2.1.3. Lung

Reutershan *et al.* [23] reported that pretreatment with isoflurane reduced acute lung injury (ALI), as evidenced by attenuation of neutrophil recruitment into lung interstitium and alveolar space, when given 1 or 12 h before endotoxin challenge or within the first hour of an already established inflammation. Other studies also showed that isoflurane pretreatment provided protective effects in sepsis [24] and I/R induced-lung injury [25]. Additionally, Zhang *et al.* [26] demonstrated that emulsified isoflurane preconditioning showed protective effects against liver and lung injury as well as improving the survival during hemorrhagic shock.

2.1.4. Kidney

Several studies have demonstrated that early preconditioning of isoflurane had protective effects against renal I/R injury [27-28]. And isoflurane delayed preconditioning provided protection against apoptosis, renal tubular damage, renal dysfunction, and renal failure after various degrees of renal I/R injury [29].

2.1.5. Liver

Isoflurane preconditioning has been shown to have

beneficial effects in variant hepatic injuries. Evidence has demonstrated that pretreatment with isoflurane protected against hepatic dysfunction and reduced mortality in septic mice [30]. Using a hemorrhagic shock model, Zhang *et al.* [26] reported that emulsified isoflurane preconditioning significantly decreased hepatic injury. Furthermore, the I/R-induced hepatic injuries were also attenuated by isoflurane pretreatment through upregulation of heme oxygenase-1 (HO-1) [31-34].

2.2. Isoflurane postconditioning

Postconditioning is an effective therapeutic strategy of attaining myocardial protection against I/R damage. Clinically speaking, postconditioning is particularly promising as no prior knowledge of the ischemic onset is needed. Isoflurane not only produces pharmacological preconditioning against myocardial I/R injury, but also exerts protective effects when administered during early reperfusion following prolonged coronary artery occlusion [35]. In a rabbit model of transient coronary artery occlusion, brief exposure to 1.0 MAC isoflurane during the final 3 min of coronary occlusion and the first 2 min of reperfusion has demonstrated to salvage myocardium from infarction and reduce the threshold of ischemic postconditioning [35]. In isolated perfused rat hearts, postconditioning by isoflurane effectively protected myocardium from I/R damage [36]. Moreover, infarct-remodeled myocardium was also protected by isoflurane postconditioning [37]. A further study demonstrated that postconditioning with intravenous infusion of emulsified isoflurane during early stage of reperfusion after a transient coronary artery occlusion induced cardiac protection in rat hearts *in vivo* as powerful as that induced by postconditioning with inhalation of isoflurane [38].

Until now, there are only several studies in cerebral protection by isoflurane postconditioning. Lee *et al.* [39] showed that isoflurane post-treatment provided neuroprotection *in vitro* study by applying OGD to rat corticostriatal slices and an *in vivo* study by subjecting rats to transient MCAO. Isoflurane administered after intestinal I/R injury can reduce intestinal injury and decrease inflammation and apoptosis while improving vascular permeability, and led to an improvement in multi-organ dysfunction [40]. However, Isoflurane postconditioning on the other organs and systems has not been extensively studied.

3. Studies on the mechanism of isoflurane on multiple organ protection

3.1. Reactive oxygen species (ROS)

Many studies reported that APC appeared to be initiated by an increase in ROS and ROS scavengers blocked APC-induced cardioprotection [41-43]. For example, Tanaka *et al.* observed that ROS scavengers abrogated isoflurane-induced APC in intact rabbits and ROS was significantly greater in myocardial nuclei of isoflurane-pretreated hearts compared with controls [44]. It is suggested that ROS is a component of the triggering process of preconditioning by anesthetics and isoflurane

preconditioning. Recent studies demonstrated that isoflurane produced ROS at complex I and III of the mitochondrial respiratory chain *via* the attenuation of complex I activity [45]. However, the precise mechanisms of ROS generation by isoflurane preconditioning need to be further investigated.

3.2. Adenosine triphosphate (ATP)-dependent K^+ (K_{ATP}) channels

In 1983, Noma *et al.* [46] identified an efflux of K^+ through K_{ATP} channels, which would be open when ATP concentrations decreased. Kersten *et al.* [47] reported that isoflurane mimicked IPC and protected myocardium against infarction *via* activation of K_{ATP} channels. Subsequent studies have confirmed the involvement of several intracellular signaling pathways in isoflurane preconditioning, and the primary target for these signals appears to be the K_{ATP} channels [48-50]. Additionally, it was observed that the mitochondrial K_{ATP} ($mitK_{ATP}$) channels, instead of the sarcolemmal K_{ATP} ($sarK_{ATP}$) channels, played an important role in the protection by isoflurane. It was further observed that the volatile anesthetics were mediated by either priming or indirectly opening the $mitK_{ATP}$ channels [51-54].

3.3. Protein kinase C (PKC)

PKC has been shown to play an important role in cardioprotection by ischemic preconditioning [55-56]. Interestingly, PKC is also considered as a major signaling component of APC [57-59], with isoforms PKC- ϵ and PKC- δ being the most relevant [60-61]. Cope *et al.* [62] first demonstrated that nonspecific PKC inhibition with chelerythrine inhibited isoflurane-induced preconditioning in the rabbit heart. The translocation of PKC isoforms was a critical step for isoflurane's cardioprotective effects against I/R injury [56, 62-63]. PKC- ϵ was targeted to the mitochondria [64] and found to be associated with many mitochondrial proteins, including components believed to constitute mitochondrial permeability transition pore (mPTP) [65]. Isoflurane-induced preconditioning resulted in a delay of mPTP opening through PKC- ϵ -mediated inhibition of mPTP opening, but not through PKC- δ [52]. These results suggested some connections between cytosolic and mitochondrial components in cardioprotection by isoflurane [52].

3.4. Phosphatidylinositol-3-kinase (PI3K)/Akt

PI3K/Akt pathway was believed to play a key role in myocardial protection by IPC [66-67]. PI3K converts phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate [68]. Phosphatidylinositol-3,4,5-trisphosphate stimulated phosphorylation of the serine-threonine kinase Akt (also called PKB) by phosphoinositide-dependent kinase 1 and subsequently inhibits formation of the proapoptotic proteins Bad, Bax and caspase 9 [69]. Activation of PI3K and modulation of the expression of pro- and anti-apoptotic proteins may play a role in isoflurane preconditioning and postconditioning in myocardial protection [70].

3.5. Extracellular-regulated kinase 1/2(Erk 1/2)

The Erk1/2 is an important member of MAPK family that plays important roles in cell differentiation, proliferation, and survival [71]. Activation of Erk1/2 has been proposed as a redundant mechanism by which downstream elements of the PI3K-Akt cascade may be stimulated to favorably modulate reperfusion injury [68]. It was demonstrated that activation of Erk1/2 mediated desflurane-induced preconditioning [72], isoflurane induced cardioprotection and ischemic postconditioning [73] during early reperfusion *in vivo* [74].

3.6. Anti-inflammatory mechanism

Nuclear factor- κ B (NF- κ B) is a key transcription factors that plays a key role in inflammatory response. Emulsified isoflurane preconditioning reduced lung injury induced by hepatic I/R, as represented by decreased levels in NF- κ B activity, tumor necrosis factor- α (TNF- α) content, and myeloperoxidase (MPO) activity, as well as a decreased level of intercellular adhesion molecule-1(ICAM-1) expression in the lung [75-76].

Lee *et al.* [28] have reported that isoflurane preconditioning ameliorated renal I/R injury in mice *via* anti-inflammatory pathway as evidenced by reduced renal influx of neutrophils and macrophages, decreased expression of proinflammatory cytokines (TNF- α , ICAM-1, and IL-1 β) as well as reduced nuclear translocation of NF- κ B 24 h after renal I/R injury. Moreover, another study also indicated that pretreatment with isoflurane provided powerful renal protection after cecal ligation and puncture (CLP) sepsis and improved survival in these septic mice. The protective mechanisms partly involved the anti-inflammatory effects of isoflurane preconditioning [30].

3.7. Nitric oxide synthase (NOS)

Nitric oxide can enhance K_{ATP} channels activation that mediates cardioprotection [77-78], and this molecule was suggested to be a critical trigger and mediator of endogenous cardioprotective signal transduction in both ischemic [79] and anesthetic-induced [80] preconditioning. At least three NOS isoforms contributing to nitric oxide production in the heart, although endothelial (e)NOS appeared to play a major role in early myocardial preconditioning [77-78]. Inhibition of NO production by the nonselective NOS inhibitor before isoflurane exposure or prolonged coronary occlusion abolished reduction in infarct size produced by this volatile agent, indicating that NO acts as an inducer and modulator of this protective process. In addition, administration of either the selective inducible (i)NOS antagonists or the selective neuronal (n)NOS inhibitor before isoflurane did not inhibit late preconditioning by the volatile agent [81]. These results suggest that eNOS but not iNOS or nNOS mediates delayed preconditioning by isoflurane.

Krolkowski *et al.* [74] demonstrated that the nonselective NOS inhibitor abolished postconditioning by isoflurane, but pretreatment with either the selective iNOS antagonist or the selective nNOS inhibitor did not inhibit postconditioning by isoflurane, leading them to suppose that eNOS mediated

cardioprotection by isoflurane during early reperfusion. Although the precise mechanism by which NOS mediates cardioprotection remains unknown, the likely mechanism appears to be through the enhancement of the generation of peroxynitrite, abrogating the Ca^{2+} overload of mitochondria [82], and importantly, inhibition of mPTP opening in response to NO generation [83-84].

In normal brain, NO exerts multiple functions including cerebral blood flow and neurotransmitter regulation [85]. Nevertheless, in ischemic brain, NO has biphasic effects depending on the amount and source of NO [85]. iNOS may be important for isoflurane preconditioning-induced neuroprotection in ischemic brain while it plays a role in the protection induced by ischemic preconditioning in brain [86] and isoflurane can induce the expression of iNOS [87].

Induction of the expression of iNOS and subsequently the overproduction of NO may be involved in the pathogenesis of ALI or acute respiratory distress syndrome (ARDS) in animals or humans during endotoxemia [88,89]. Li *et al.* [90] showed that isoflurane preconditioning attenuated ALI induced by lipopolysaccharide (LPS) in rats and the process may be mediated partly via inhibition of endogenous iNOS-NO axis activation. However, Pang *et al.* [91] reported that pretreatment with isoflurane attenuated inflammatory process in the lung tissue of rats with LPS-induced ALI, and this preconditioning effect was mainly mediated by activation of endogenous iNOS in the lung. The different role of iNOS pathway in pulmonary protection needs to be further investigated.

3.8. p70S6K

p70S6K is an important regulator of protein translation, which is activated following phosphorylation by PI3K-Akt or Erk 1/2. PI3K-Akt- or Erk 1/2-induced activation of p70s6k may contribute to preservation of myocardial integrity during reperfusion by inactivating Gsk-3 β and inhibiting apoptotic cell death [92]. Krolikowski *et al.* [74] showed that administration of the p70s6k inhibitor rapamycin during coronary artery occlusion abolished reductions in infarct size produced by isoflurane during early reperfusion.

3.9. Janus kinase -signal transducer and activator of transcription (JAK-STAT)

The JAK-STAT pathway plays a vital role in gene transcription by transducing signals received at the cell surface to the nucleus [93]. This pathway can be activated by stressors such as ischemia, mechanical stress, cytotoxic agents, or bacterial inflammation. Previous studies demonstrated that activation of JAK-STAT pathway had cardioprotection against ischemia [94-95]. It was also demonstrated that the observed cardioprotective effects afforded by postconditioning with inhalation of isoflurane or infusion of emulsified isoflurane were mediated by activation of JAK-STAT pathway [38]. In addition, "crosstalk" between JAK-STAT pathway and other cell signaling pathway, such as PI3K-Akt, in cardiac preconditioning or postconditioning has been confirmed in different models [96].

3.10. Anti-apoptosis

Apoptosis is a fundamental process of cell death that occurs via activation of distinct signaling pathways involving mitochondria and caspases. Studies have demonstrated that the inhibition of apoptosis reduced infarct size and attenuated cardiac dysfunction following myocardial infarction [97-98]. It was demonstrated that isoflurane-induced preconditioning attenuated myocardial apoptosis in rabbits after regional ischemia and reperfusion via modulation of B cell lymphoma protein-2 (Bcl-2) family proteins. Emulsified isoflurane suppressed ischemia-induced myocardial apoptosis by modulation of the expression of pro- and anti-apoptotic proteins, specifically Bax, caspase 3 and Bcl-2 [99].

The anti-apoptotic protein, Bcl-2, attenuates cellular injury by inhibiting mitochondrial cytochrome *c* release into the cytosol [100]. Wang *et al.* [101] demonstrated that Bcl-2 mediated isoflurane-induced and ischemic postconditioning against myocardial ischemic injury by indirectly modulating mPTP activity in rabbits. Moreover, inhibition of the apoptotic protein p53 lowered the threshold of isoflurane-induced cardioprotection during early reperfusion via mPTP dependent mechanism *in vivo* [102].

Neuronal cell death plays a role in cerebral ischemia. In the ischemic penumbra of rat brain, pretreatment with isoflurane increased Bcl-2 expression and reduced cytochrome *c* release from the mitochondria [103]. Gwak *et al.* [104] also demonstrated OGD reduced Bcl-2 expression and that isoflurane preconditioning preserved Bcl-2 expression level in OGD-treated cells. In addition, HA14-1, a specific Bcl-2 inhibitor, abolished isoflurane preconditioning-induced neuroprotection in the neuronal cultures [104].

3.11. Glutamate

Glutamate is both a major excitatory neurotransmitter and a potent neurotoxin at high concentrations (micromolar levels). It plays a major role in the initiation and evolution of ischemic brain injury [85]. Ischemia-induced glutamate accumulation and the consequent glutamate neurotoxicity contribute significantly to cell death by overstimulating glutamate receptors [105, 106]. In rat cerebellar slices, isoflurane preconditioning has been shown to be a potent neuro-protection against glutamate neurotoxicity in a PKC and NOS-dependent mechanism [106]. Addition of a specific glutamate transporter inhibitor during OGD blocked any benefits of isoflurane preconditioning [107].

3.12. HIF-1 α

HIF-1 α is a heterodimer and acts as a transcription factor that has been shown to be involved in hypoxic and ischemic preconditioning in several tissues. Interestingly, HIF-1 α is also involved in anesthetic-induced organ protection. It has been shown that isoflurane preconditioning decreased myocardial infarction size via up-regulation of HIF-1 α in rabbits [108]. One possible mechanism of APC is the activation of HIF-1 α which in turn activates the expression of its target genes, such as vascular endothelial growth factor (VEGF) [109].

3.13. Mitochondrial (m)Ca²⁺ overload

Recent evidence suggested that the signaling pathways converge at the mitochondria and preserve their function, resulting in the cardioprotective effects afforded by preconditioning [110-111]. Cardiac I/R injury was associated with mCa²⁺ overload [112]. During reperfusion, the interplay between excess mCa²⁺ overload, ROS production, and mPTP opening, was the crucial mechanism of cell injury. Ca²⁺ influx may contribute to the underlying cardioprotective signaling cascade, while its attenuation will reduce reperfusion injury. Isoflurane-induced preconditioning triggered persistent changes in the inactivation of cardiac L-type Ca²⁺ channels and prevented or delayed apoptosis [113-115].

3.14. Others

Caveolae are small, flask-like invaginations derived from the plasma membrane. Three isoforms of caveolin, Cav-1, -2, and -3, are involved in the formation of caveolae and interact with signaling molecules via a scaffolding domain [116]. Cav-1 and Cav-3 are essential for early isoflurane preconditioning induced cardiac protection [117-119].

O-linked β -N-acetylglucosamine (O-GlcNAc) is a posttranslational glycosylation modification of nucleocytoplasmic and mitochondrial proteins and is analogous to phosphorylation or acetylation of proteins [120]. More than 80 different proteins, including transcription factors, kinases and phosphatases, are reversibly modified by O-GlcNAc, which is necessary for cell survival [121]. Zachara *et al.* [122] reported that multiple stimuli increased cellular concentrations of O-GlcNAc in mammalian cells. Blockade of this response will decrease cell survival whereas increases in O-GlcNAc levels will maintain cell viability [122]. Studies indicated that O-GlcNAc may also be associated with IPC of cardiac myocytes [120,123].

The ubiquitin-proteasome system is the major nonlysosomal system for degrading proteins within cells [124]. Ischemia can induce the accumulation of ubiquitinated proteins in damaged brain regions, and blockade or overactivation of the ubiquitin-proteasome system can result in cell malfunction and death [125-126]. It was indicated that isoflurane preconditioning significantly eliminated neuronal injury through a decrease in accumulation of ubiquitin-conjugated proteins after ischemia in a global cerebral ischemia mouse model [127].

A novel class of K⁺ channels, two-pore-domain potassium channels (K2P channels) are known as “back-ground” or “baseline” K⁺ channels because they are open at membrane potentials over the whole voltage range that includes resting membrane potentials and they help restructure depolarizing influences [128]. TREK1 channels, as one member of K2P channels, are highly expressed in the brain and spinal cord [129], and they can be obviously activated by isoflurane [130]. Additionally, Yin *et al.* [22] showed that isoflurane-induced delayed preconditioning against spinal cord I/R injury may be mediated through the TREK1 pathway.

4. Summary

Isoflurane preconditioning and postconditioning may produce multi-organ protective effects and involve a variety of potential mechanisms. Although only cardioprotection by isoflurane preconditioning has been used in clinical practice, most research results still derive from animal experiments *in vivo* or *in vitro*. The emphasis of further research should be focused on the roles of different mechanisms in the protective process. Such studies will help to explore the exact mechanisms and translate experimental findings into clinical practice.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (30972856, 81071752, 81071067 and 81170321), and by Jiangsu Province's outstanding Medical Academic Leader program (LJ201124).

Conflict of interest: None declared.

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